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Combined Method of Ultrasound Therapy of Oncological Diseases

A. L. Nikolaev[a], A. V. Gopin[a], V. E. Bozhevol'nov[a], H. M. Treshalina[a], N. V. Andronova[a], I. V. Melikhov[a], D. V. Filonenko[a,c], S. E. Mazina[a], G. K. Gerasimova[a†], E. V. Khorosheva[b], I. N. Mikhailova[b], L. V. Demidov[b], B. Yu. Bokhyan[a], B. Ya. Kogan[d], and O. L. Kaliya[d]

[a] *Lomonosov Moscow State University, Moscow, 119991 Russia*
e-mail: nicmsu@gmail.com

[b] *N.N. Blokhin Russian Cancer Research Center, Moscow, Russia*

[c] *Moscow City Cancer Hospital no. 62, Moscow Health Care Department, Krasnogorskii raion, Stepanovskoe, Moscow oblast, Russia*

[d] *Research Institute of Organic Intermediates and Dyes, State Scientific Center, Federal State Unitary Enterprise, Moscow, Russia*

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Abstract—The experience of the joint research by the Department of Chemistry, Lomonosov Moscow State University, and the Federal State Budgetary Scientific Institution "N.N. Blokhin Russian Cancer Research Center" (FSBSI "N.N. Blokhin RCRC"), on the application of medium-intensity ultrasound in combination with chemotherapy and sonosensitizers in the treatment of cancer diseases was summarized. A cycle of preclinical trials showed that the method allows enhancing the damaging effect of ultrasound on the tumor, while no metastasis-promoting and toxic effects are exerted. The combined method is being currently tested in clinical trials.

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INTRODUCTION

The first observations of ultrasound action on malignant tumors date back to the early 1930s [1]. Lasted until the 1950s, scattered attempts of clinical application of ultrasound in treatment of malignant neoplasms had led to a general disappointment, and this direction "decayed" until the mid-1970s, when oncologists began to widely implement thermotherapy in the form of the so-called hyperthermia, which implies an increase in body temperature, or local temperature, of the patient directly in the tumor lesion [2–4]. Diathermy, inductothermy, and microwaves used as sources of heat suffer from several drawbacks. In particular, they do not allow accurate calculation and monitoring of temperature in deep tumor localization zones [3]. In this respect, ultrasound offers an advantage of acoustic wave focusing with release of a maximum energy, in particular, of thermal energy focusing. The temperature increase accompanying the ultrasound application can reach tens of degrees, whereby the biological objects and their functional characteristics can be significantly affected [5–11]. A specific feature of ultrasonic heating of biological tissues consists in that, beyond a certain ultrasound intensity, the thermal effects from acoustic action are accompanied by nonlinear effects characteristic for ultrasound solely.

Also, ultrasound exerts a biological action through mechanical effects as determined by the ultrasonic amplitude parameters (vibrational displacement, vibrational velocity, acceleration, sound pressure, gradient between the sound pressure maximum and minimum, radiation pressure). The mechanical effect of ultrasound is sharply enhanced when ultrasonic cavitation arises in a biological medium [6–8, 12–23]. Cavitation is effective toward energy concentration and power transformation. In biological media, cavitation can cause changes in cells, in particular, due to microfluxes and radiation forces associated with vibrating gas

† Deceased.

inclusions. These forces cause formation of vortices in the vacuoles, as well as deformations and displacements of intracellular structures and cytoplasm, eventually leading to destruction of the tumor tissue [24].

Since recently, the possibility for precisely delineating the tumor borders using computed tomography techniques has existed, and the ultrasonic therapeutic equipment itself has improved. This has given a new lease of life to acoustic cancer therapy, which is now competitive with radiation therapy traditionally applied in oncology [25–28].

Several lines can be conditionally distinguished in ultrasound therapy of malignant neoplasms [29].

(1) Thermal tumor destruction by unfocused ultrasound beam at a high frequency (1–2.5 MHz) with a medium ("therapeutic") intensity (up to 2 W cm^{-2}) and sufficiently long exposure time, with repeated treatment sessions.

(2) Noninvasive surgery (High Intensity Focused Ultrasound, HIFU) involving application of high-intensity (100–10000 W cm^{-2}) focused ultrasound to reach temperatures above 60°C, whereby instantaneous irreversible protein denaturation and tissue necrosis are caused.

(3) Ultrasonication combined with chemotherapy, in which case the main effect from the ultrasound exposure consists is enhanced bioavailability of chemotherapeutic agent to the pathological site.

(4) Ultrasonic destruction (5–7 W cm^{-2}) of drug carriers (liposomes, neutral for the body and subsequently absorbable polymer capsules, or anticancer drug-loaded erythrocytes) [30] directly in the pathological site area.

(5) Sonodynamic therapy [31, 32], which consists essentially in exposure of the tumor to ultrasound combined with nonmedicinal chemical compounds, sonosensitizers ("sonodynamic" is the term used by analogy with the commonly accepted "photodynamic therapy" term).

When applied independently, ultrasound holds promise for cancer treatment only as a method in which focused acoustic beams of high intensity (≥1000 W cm^{-2}) are used. This method (HIFU) seeks to achieve two closely interrelated major objectives: local tissue destruction and control of the accuracy of ultrasound in targeting a specific body area. The HIFU surgery method is being gradually introduced into clinical practice. The disadvantages of the method include expensive precision equipment to be serviced by trained personnel. Also, its application is advisable not for all types and localizations of cancer formations.

Being much simpler in terms of both implementation and operation, the method of sonodynamic therapy of malignant diseases in some cases may be considered as an alternative to HIFU surgery. The useful ultrasound intensities lie in the 1–10 W cm^{-2} range. The focusing is of less importance than in HIFU surgery. The selectivity of action is provided by several factors: selective accumulation of sonosensitizer in the tumor, predominant action of ultrasound on the tumor focus, and predominant ability of healthy tissue to recover. The mechanism of therapeutic action still remains debatable, but there exist good reasons to suggest its free-radical nature associated with the cavitation produced by ultrasound.

The currently available data mostly suggest that sonodynamic therapy is targeted at the outer cell membrane (plasmalemma), whereas the intracellular structures, the core with the genetic apparatus and cellular organelles, undergo only secondary damage.

Given the multiplicity of the factors of ultrasonic action on biological objects, it can be presumed that a single action mechanism does not exist for sonosensitizers.

In application of ultrasonic methods, the accuracy in hitting the target is determined by the wavelength: the shorter the wave, the higher the hitting accuracy. At the same time, a decrease in the wavelength causes the penetrating power of radiation to decrease. The balance between the required accuracy and the depth of penetration of ultrasound determines the optimum irradiation parameters.

A major problem encountered with application of sonodynamic therapy is the control of localization of the main factor destroying the tumor, the cavitation processes in unfocused acoustic fields. This problem can be solved by producing a local frequency-dependent increase in the effective ultrasonic absorption coefficient via introducing into the medium of aggregates of nano- and microparticles of specific nature with specific size distribution. The therapeutic effect from ultrasound exposure of biopolymers modified with nanoparticle aggregates is achieved through release of additional acoustic energy at the localization sites of these aggregates. The reason is a substantial change in the response of the system to ultrasound exposure, caused by the aggregates whose

physicochemical properties differ from those of biopolymer structures [33]. Specifically, they act as peculiar transducers of acoustic energy, which produce local changes in the ultrasonic absorption coefficient. The nano- and microinclusions ensure localization of acoustic energy in a volume whose size is mainly determined by the dimensions of the aggregates and can be significantly smaller than the ultrasound wavelength scale. If the aggregates are localized in the tumor cells, the release of additional acoustic energy therein causes the tumor cells death or growth inhibition.

Based on the above considerations, a method of tumor destruction by exposure to ultrasound in the presence of solid nanoparticles and their aggregates was developed. We assign the "solid-phase sonosensitization" term to the phenomena underlying this method and attributable to the presence of the solid phase, and the "solid-phase sonosensitizers" term, to the nanoparticles and their aggregates.

Currently, it is difficult to quantitatively assess the contributions made by different factors to the additional "destructive potential" of ultrasound, associated with the presence of solid sonosensitizers. Presumably, the most important factors are as follows:

– physical destabilization of cellular structures, leading to enhanced sensitivity to shear stresses [34];

– thermal effects influencing the mechanical strength and permeability of biological membranes [35];

– mechanical destruction of biomembranes by nanoparticles [36]; and

– local decrease in the cavitation strength of the medium, leading to an increase in the intensity of cavitation processes [14].

All the above-mentioned factors are likely to be interrelated and to be producing a cross-cutting action. The main objective to be achieved with solid-phase sonosensitization is to ensure that the solid-phase inclusions will be localized in the lesion. There exist two fundamentally different ways of their delivery: introduction into the bloodstream of already synthesized nanoparticles and synthesis of nanoparticles directly in the lesion [37]. Formation of nanoparticles and their aggregates directly in the tumor is afforded by the biochemical features of the tumor growth whose characteristics are collectively referred to as atypia. The possibility of using metabolic atypia for selectively enhancing the tumor sensitivity to the action of radiation and thermal agents that do not have a pronounced specificity was indicated as early as 1970s by Shapot [38] and von Ardenne and Reitnauer [39].

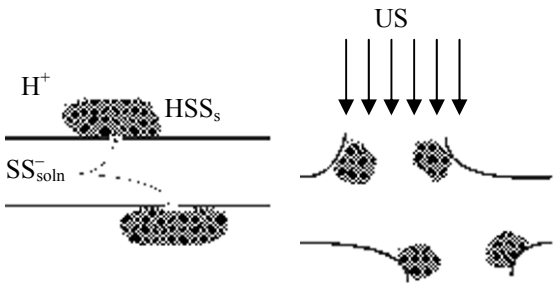

Fig. 1. Scheme of the ultrasound (US)-induced destruction of the tumor modules at the localization site of the sonosensitizer solid phase (HSS) aggregates.

Owing to metabolic atypia, the physicochemical conditions in the tumor (low pH, high content of calcium ions in the extracellular fluid, monotonous lipid membrane structure) differ from those in normal tissues surrounding the tumor. These differences allow formation of a solid phase predominantly in the tumor via precipitation after intravenous injection of solutions of compounds whose calcium salts or acid forms are insoluble under the tumor conditions (Fig. 1). Thereby, it is possible to achieve selective formation of nanoparticles and their aggregates predominantly in the tumor, taking advantage of the least specific and therefore most stable manifestations of its atypia. Subsequent to the solid phase formation, ultrasound action provides for selective destruction of the tumor structures.

Here, we present an approach to cancer therapy in which the exposure to medium-intensity ultrasound is combined with the action of noncytotoxic substances, enhancing its effect, and with that of chemotherapeutic agents. The implementation of this approach involves development of methodologies for selection and synthesis of optimal sonosensitizers (soluble or in the nanoform), designing equipment for preclinical and clinical trials, and devising optimal therapeutic schemes for clinical application, as well as incorporation of the method into clinical practice.

The above-listed activities were performed along several lines simultaneously: laboratory physicochemical studies and in vitro and in vivo experiments.

Sttudies on Model Gel Systems

The main lines of the physicochemical studies were as follows:

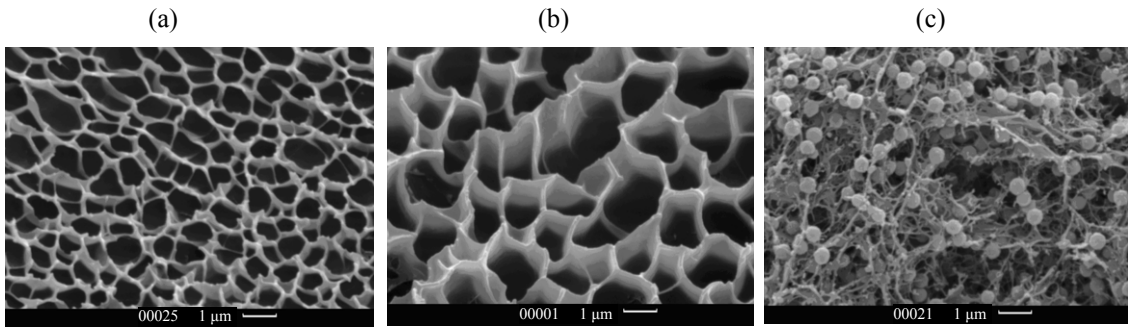

Fig. 2. Electron micrographs of the hydrogel samples: (a) unmodified and (b, c) modified with (b) iron(III) hydroxide, and (c) calcium salt of Teraphthal.

– elucidate how the polymer matrix and the synthesis conditions influence the solid-phase modifier localization and morphology;

– evaluate the thermal and cavitation acoustic effects in the modified gel systems; and

– determine the optimum ultrasound exposure regimes.

The studies were carried out on model gel systems, polyacrylamide and agarose hydrogels, imitating the tumor tissue. They are well-suited for development of a synthesis methodology for solid-phase sonosensitizers, as well as for preliminary evaluation of the thermal and cavitation effects in acoustic fields of different frequencies and intensities.

As solid-phase sonosensitizers served iron(III) hydroxide, barium sulfate, derivatives of cobalt octa-4,5-carboxyphthalocyanine octasodium salt (Teraphthal), silica gel, hydrophobized silica, and hydroxyapatite.

Teraphthal (developed by the Research Institute of Organic Intermediates and Dyes, State Scientific Center, Federal State Unitary Enterprise), a component of the catalytic system used for malignant tumor therapy, is nontoxic and highly soluble in water [40, 41]; the calcium and acid forms of Teraphthal are insoluble. Among the above-listed solid-phase sensitizers, only Teraphthal and its derivatives and hydroxyapatite can basically be considered promising for clinical application. Other sensitizers were used by us for confirming the general character of the trends revealed in our study.

The preliminary choice of ultrasonic treatment modes was based on a comparative evaluation of the effect produced by ultrasound exposure on the dynamics of pathomorphosis of the tumor and healthy tissue. The ultrasound intensity was limited by the maximum value at which no irreversible changes in the healthy tissue were detected.

The sonosensitizers were synthesized by two routes: by counter-diffusion of the reactants whose interaction in the preliminarily synthesized gel [Teraphthal, iron(III) hydroxide] gives a poorly soluble compound and by introduction of solid-phase sonosensitizers during synthesis of the gel (calcium salt of Teraphthal, silica gel, silica gel, hydrophobized silica gel, and hydroxyapatite) [42, 43]. The difference between these routes lies mainly in the nature of bonding between the solid-phase sonosensitizer and the polymer matrix. In the former route, the polymeric matrix threads can participate in nucleation, growth, and aggregation of the sonosensitizer crystals to which they get closely connected and whose shape and size distribution they determine. In the latter route this possibility is minimized, since the already formed crystals are introduced into the gel system being formed.

In the counter-diffusion route, formation of sonosensitizers under conditions of metabolic atypia of the tumor tissue from soluble precursors is simulated. The route with introduction of solid-phase sonosensitizers during the gel synthesis allows preparation of gels containing inclusions of any nature with the desired size distribution and to some extent simulates the way by which the already synthesized solid-phase sonosensitizers are introduced into the bloodstream.

Influence of the Polymer Matrix on Localization of Solid-Phase Modifiers

The influence of the polymer matrix on the phase formation processes was evaluated by scanning electron microscopy, X-ray diffraction analysis, and differential scanning calorimetry, and for iron(III) hydroxide, by Mössbauer spectroscopy.

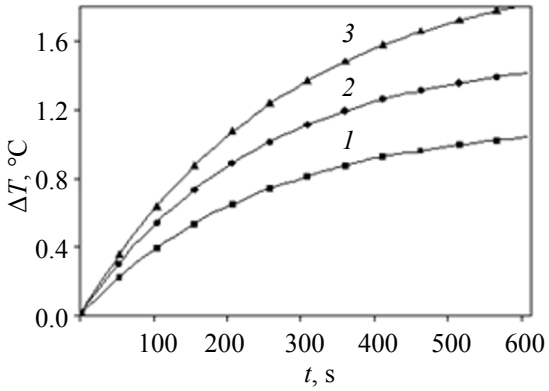

Fig. 3. Dynamics of temperature rise for the agarose hydrogel samples in the ultrasonic field (2.64 MHz, 1 W cm^{-2}). Sample modified with: (*1*) iron(III) hydroxide and (*2, 3*) calcium salt of Teraphthal obtained by (*2*) mixing and (*3*) counter-diffusion route.

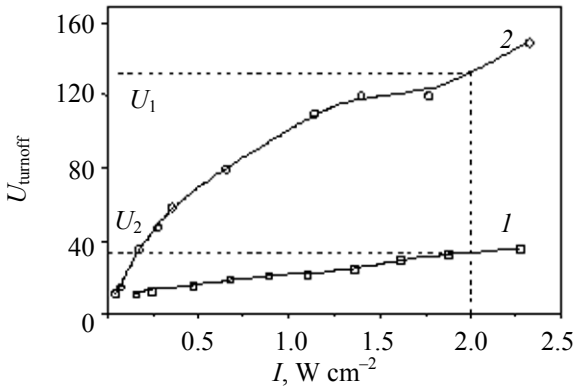

Fig. 4. Intensity of the cavitation processes, arb. units, in the agarose hydrogel as a function of the ultrasound intensity *I* (frequency 0.88 MHz): (*1*) unmodified hydrogel and (*2*) hydrogel modified with calcium salt of Teraphthal.

Our experimental data suggest the presence of at least two types of solid phase crystallizing in hydrogels, of which one solid phase is uniformly distributed over the polymer matrix threads and the other is localized on its individual centers, which act as the solid phase nucleation sites. Iron(III) hydroxide is an example of solid phase of the first type, and calcium salt of Teraphthal, of the second type (Fig. 2).

Acoustic Effects in Solid-Phase-Modified Gels

The presence of two types of solid-phase localization in gels suggests that acoustic effects in such systems may differ significantly. Figure 3 shows the dynamic curves of ultrasound-induced heating of the modified gel samples (2.64 MHz, 1 W cm^{-2}); the modifiers used are calcium salt of Teraphthal, both that obtained by counter-diffusion of the reactants and that introduced during the gel synthesis, and iron(III) hydroxide, prepared by the counter-diffusion route.

The acoustic effect was the most pronounced in the case of systems in which sonosensitizers, obtained by counter diffusion of the reactants, are localized on individual elements of the matrix volume. The iron(III) hydroxide nanoparticles distributed in the polymer matrix network exhibit even a small "negative" thermal acoustic effect, probably associated with increased rigidity of the polymer matrix due to encrusting with a finely-dispersed precipitate. These findings suggest that an important role in manifestation of the acoustic thermal effects is played by the character of interaction of the sonosensitizer with the gel matrix, which in turn is determined by their chemical nature.

Comparative assessment of the cavitation properties of the gels with and without impurities was carried out with the use of an IS-3MS cavitation indicator whose operation is underlain by analysis and processing of the cavitation noise spectrum. The experimental results (Fig. 4) indicate that the signal level recorded at the ultrasound intensity of 2 W cm^{-2} for the modified hydrogel sample (U_1) significantly (more than 4 times) exceeds that recorded for the gel without modifier (U_2).

Similar evidence for enhancement of the cavitation processes in the presence of the modifier (hydrophobized silica gel) was gained from the molecular-weight distribution data for Pluronic F127 polymer subjected to ultrasound exposure. In the presence of the modifier, the molecular-weight distribution is shifted to lower molecular weights, thereby indicating degradation of the polymer molecules. At the same time, the molecular-weight distribution of the unmodified polymer subjected to ultrasound exposure remains unchanged.

Ultrasound Exposure Regime

The main task of combined sonodynamic therapy consists in reducing the ultrasound exposure dose while reaching the full therapeutic effect. Studies on the model gel systems modified with solid-phase inclusions showed that this task can be accomplished in two ways: by simultaneous exposure of the tumor to ultrasound of two frequencies (0.88 and 2.64 MHz) and by using ultrasound in pulsed mode. A combination of frequencies allows increasing the efficiency of the cavitation processes and, if desired,

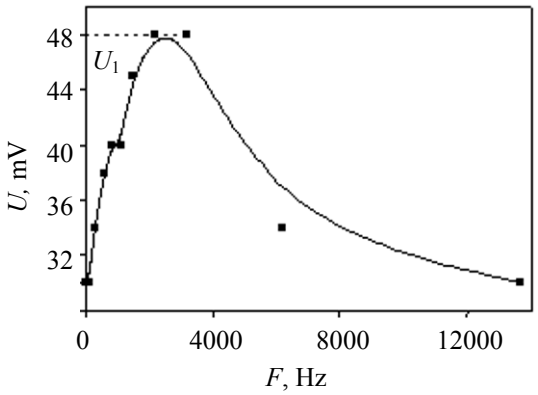

Fig. 5. Intensity of the cavitation processes in the gel system as a function of the modulation frequency F (duty factor 2, ultrasound frequency 0.88 MHz, ultrasound intensity 2 W cm^{-2}).

separating the cavitation and thermal effects. At the ultrasound frequency of 0.88 MHz, cavitation is observed, and a relatively small increase in temperature is achieved; at the frequency of 2.64 MHz, fairly intense heat evolution is observed, and no cavitation is detected.

Our experiments on the muscle tissue models showed that, by varying the ratio of the intensities of ultrasound with the frequencies used, the temperature of the object irradiated can be decreased by 1.5–2°C at the same acoustic energy dose. This finding is essential for cases where the toxicity of the effect is determined by the temperature in the tumor.

It was found that, when exposed to pulsed ultrasound (U_1 = 48 mV, Fig. 5), the unmodified gel samples and those modified with the solid-phase sonosensitizers exhibit a higher maximum signal associated with intensity of the cavitation processes than does the gel without sonosensitizers under exposure to continuous-wave ultrasound (U_2 = 34 mV, Fig. 4). The optimum modulation frequency depends on the gel size and density. In the current stage of research, this finding cannot be explained unambiguously. A certain role in this effect may be attributed to the resonance phenomena determined by the parameters of the gel as a whole.

Thus, an increase in the effective ultrasonic absorption coefficient and enhancement of the local heat evolution can be achieved through combining the use of solid-phase modifiers with exposure to the "cavitation" frequency of 0.88 MHz in the pulsed mode and to the "thermal" frequency of 2.64 MHz in the continuous-wave mode. The solid-phase inclusions will provide for localization of the effects and reduction of the cavitation strength of the adjacent area; the ultrasound exposure in the pulsed mode allows reducing the total dose of ultrasonic irradiation while reaching the full therapeutic effect, and ultrasound exposure at the frequency of 2.64 MHz provides for the necessary heating of the lesion.

Optimization of the Phase Formation Conditions

Optimization of the therapeutic scheme based on combining ultrasound with sonosensitizers requires the knowledge of how the initial concentration of soluble solid-phase precursors and the time interval between their introduction and the formation of a solid-phase modifier are related to the total mass of the resulting solid phase and its spatial and particle size distributions.

We developed a mathematical model describing the dynamics of accumulation of the modifier (sonosensitizer) mass in a porous matrix under conditions relatively close to those of phase formation in vivo, i.e., when the concentration of one component in tumor (A) is constant and that of the other component introduced into the blood stream (B) decreases in time by an exponential law [44]. The equation describing the formation of a solid-phase modifier appears as follows:

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AB \xrightarrow{\beta_0, \beta_1} AB^{S},$$

where k_1 and k_2 are the rate constants of the forward and backward homogeneous reaction, and β_0 and β_1, solid phase nucleation and growth constants, respectively; index "S" refers to the solid phase.

With relevant database available, calculations allow determining the time interval between the introduction of the sonosensitizer and the ultrasound exposure, at which the most effective ultrasonic action is produced on the tumor. It should be noted that creation of such database poses a number of problems associated with adapting multiparameter kinetic schemes to complex biological systems. In the current stage, and possibly in the future, the choice of practically significant parameters of ultrasonic therapeutic schemes will most likely be made on the basis of experimental data.

The main consequences of the physicochemical examinations of the acoustic effects on the model gel systems to be taken into account by in vitro and in vivo studies are as follows:

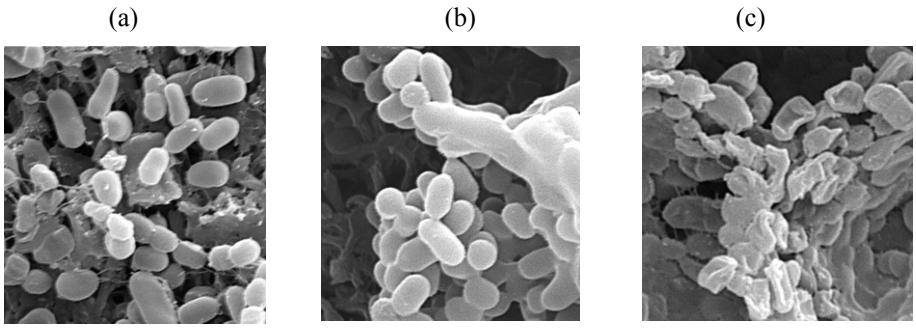

Fig. 6. Combined effect of ultrasound and Teraphthal on *Enterococcus spp.* bacteria: (a) native bacteria, (b) bacteria after ultrasound exposure, and (c) bacteria exposed to the combined action of ultrasound and Teraphthal. Teraphthal concentration 10^{-5} M, ultrasound parameters 0.88 MHz, 1 W cm^{-2}, exposure time 10 min.

– introduction of nanoinclusions into the polymer matrix causes significant changes in the cavitation threshold and intensity and in the amount of the heat evolved;

– dual-frequency ultrasound exposure combined with the pulsed mode extends the range of therapeutic capabilities of ultrasound; and

– pulsed ultrasound regimes can be effective toward reduction of the total ultrasonic dose and possible appearance of "resonance" effects.

In Vitro Studies

Seeking the optimum regimes of sonodynamic cancer therapy is a very complex task which, in the absence of a clear algorithm, can only be solved via applying general considerations and using the findings from related fields. Considering the difficulties of performing in vivo experiments, we carried out the basic experiments in cell cultures and bacteria in parallel.

The universal nature of cellular responses to external stimuli makes promising the application of cells as models for obtaining information about both the optimum regimes and the physicochemical and physiological mechanisms of the effects examined. Also, such models allow additionally assessing the cell viability and cell metabolism in experiments on transplantation of cells after in vivo exposure.

Cancer cells have some similarity to bacterial cells in the structural and functional characteristics, including structural features of the cytoplasmic membrane, its increased permeability, transition to glycolysis, etc. This similarity predetermined the choice of *Enterococcus spp*. bacterium as an alternative model for assessing the biological effect produced by ultrasound in combination with sonosensitizer Teraphthal. This bacterium is adapted to existence under the human body conditions at mesophilic temperatures and neutral pH, exhibits high proliferative activity, and is easily cultivated on nutrient media. Bacteria have mucosa, which can be considered as an analog of the medium surrounding the cancer cell inside the tumor.

The synergism of ultrasound and Teraphthal, identified for Ehrlich ascites tumor cell suspension, as paralleled by an increase (2.5 times) in the level of intracellular free radicals, makes Teraphthal a promising agent for sonodynamic therapy.

The advantage offered by ultrasonication in the pulsed mode, which demonstrated its efficacy with gel systems, was also confirmed for cells. This is especially relevant to the effect of pulsed regimes on the ability of certain malignant strains to develop multidrug resistance, which is limit the acheives in chemotherapy results.

Experiments using human erythroleucemia cell culture K562 with induced resistance to doxorubicin de-monstrated that, under the action of ultrasound in the continuous-wave mode, cells can restore their sensitivity to doxorubicin in three cell generations. Upon pulsed mode exposure at ultrasound frequency of 2.64 MHz, modulation frequencies of 1240–225–20 Hz, duty factor of 2, and Teraphthal concentration of 10^{-5} M, to increase the drug sensitivity of the cells in 100–50–25 times, accordingly. Teraphthal allows restoring the drug sensitivity of the cells and optimizing the effect by varying the ultrasonication regimes [45].

Figure 6 shows the electron-microscopic data on the morphology of the Enterococcus bacteria cells exposed to ultrasound and Teraphthal separately and in combination. The ultrasound exposure causes destruct-

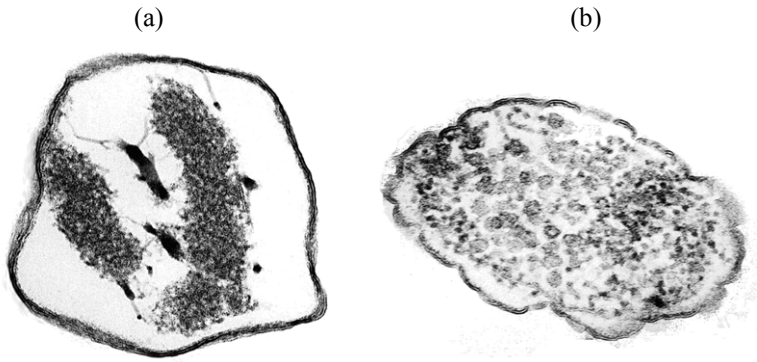

Fig. 7. Electron micrographs of *Enterococcus spp.* cell section: (a) native bacteria and (b) bacteria exposed to the combined action of ultrasound and Teraphthal.

tion of a part of the bacteria, as accompanied, apparently, by outflow of cytoplasm. However, most of the cells preserve their normal form. The bacteria processed with Teraphthal exhibit changes in the shape and destruction of membranes in virtually all the cells processed. A similar pattern is demonstrated by the transmission electron-microscopic images of the cell sections (Fig. 7). It is seen that the sonodynamic action of Teraphthal with ultrasound causes destruction of the cell membranes and organelles.

When introduced into a suspension of bacterial cells, Teraphthal gets completely bound to the cells, as evidenced by the fact that its concentration in the centrifugate decrease virtually to zero. Presumably, in a biological system, Teraphthal forms an insoluble calcium salt on the membrane structures of the bacterial cells. This leads to intensification of the cavitation processes at the localization site of the solid-phase inclusions upon application of an ultrasonic field, resulting in cell destruction and death. It was found reproducibly that, when in 10^{-4}–10^{-5} M concentrations, Teraphthal causes the cells survival rate in an ultrasonic field to noticeably decrease at ultrasound intensities of 0.6–2.0 W cm^{-2}.

The results obtained give us a reason to consider studies combining the experiments on cells and bacteria as consistent, complementary, and suitable for preliminary assessment of the efficacy of sonosensitizers and optimization of the ultrasound exposure regimes. The main consequence of the results obtained in the combined action studies is that Teraphthal acts as an efficacious sonosensitizer under ultrasound exposure of biological structures and that pulsed ultrasound efficiently overcomes multidrug resistance. Furthermore, the experiments on bacteria showed that membrane structures are the main targets for ultrasound exposure combined with sonosensitizer action.

On the whole, our experiments on cell cultures and bacterial cells confirmed the main findings from the solid-phase sonosensitization studies on model gel structures.

Preclinical Investigation

Federal State Budgetary Scientific Institution "N.N. Blokhin Russian Cancer Research Center" (FSBSI "N.N. Blokhin RCRC") jointly with the Department of Chemistry, Lomonosov Moscow State University, conducted comprehensive preclinical studies of sonodynamic therapy on the different intramuscular transplanted prognostic significant murine tumor models: Lewis lung cancinoma (LLC), B16/F10 melanoma, Cholangiocellular cancer RS-1, and alenocarcinoma Ca755.

Ultrasound exposure conditions were as follows: dual-frequency ultrasound exposure (0.88 MHz + 2.64 MHz), total intensity ≤ 5 W cm^{-2}, and total area overlapped by acoustic stream ~3 cm^2.

As sonosensitizers lacking cytotoxic activity served Teraphthal and its derivatives, as well as octasodium salt of zinc octacarboxyphthalocyanine and nano-hydroxyapatite.

The treatment efficacy was estimated from the tumor growth dynamics in control and experimental groups. The animals were ranked in groups according to the tumor volume. After ultrasound exposure, at regular time intervals (t) the average tumor volumes were calculated for the experimental (V_t) and control (V_0) groups. Next, the tumor volume doubling time τ was determined from the $V_t/V_0 - t$ dependence, after which the enhancement ratio for the antitumor effect

from the combined exposure K was calculated as the ratio of τ in the experimental group to that in the control group (n = 6–10). For drugs possessing with significant antitumor activity the tumor volume doubling times under the action of cytotoxic drugs, ultrasound, and an ultrasound + cytostatic drug combination were compared. Statistical processing of the data was performed using the Fisher-Student test.

Sonodynamic Therapy Conditions: Selection of Solid-Phase Sonosensitizers

The specific experimental conditions for ultrasound therapy of transplanted tumor in animals are elected with the view of achieving the optimal combination of two types of effects, a therapeutic effect and an adverse effect, which in turn depends on numerous interrelated parameters of the therapeutic scheme. These parameters include: the frequencies of the ultrasonic fields combined and their corresponding intensities; the initial tumor volume; the contact medium temperature; the sonosensitizer dose; the time interval between the sonosensitizer injection and the ultrasound exposure; the exposure time; the presence of an additional enhancing factor, in particular, cytostatic agents; and the number of courses of therapy.

As mentioned above, the ultrasound exposure parameters were selected on the basis of the data on pathomorphosis dynamics to the intent that injuries to healthy tissues be prevented. The experimental tumors ranged in volume from 1 to 2 cm^3.

The temperature of the contact medium at fixed ultrasound exposure parameters determined the temperature in the tumor: Its lower limit was the temperature of the animal body, and the upper limit, the temperature at which adverse irreversible effects were observed under the therapeutic scheme conditions.[1]

The sonosensitizer dose, the time intervals between the sonosensitizer injection and the ultrasound exposure, and the exposure time were determined experimentally.

Specific therapeutic schemes were chosen with the view to prospective clinical application.

The experiments in which the effect of ultrasound was combined with that of intravenous showed that the optimum time interval between the sonosensitizer injection and the ultrasound exposure is 1 h, and the treatment efficacy tends to increase with increasing dose. However, toxic effects tend to increase with increasing dose as well. For example, mice in the groups exhibited a close to 50% mortality at the dose of 80 mg/kg.

The following conditions were selected for Teraphthal application: the time interval between the sonosensitizer injection and the ultrasound exposure 1 h, dose range 20–40 mg/kg, exposure time 7–10 min, and tumor temperature 39–41°C. It should be noted that the 1-h interval corresponds to the time it takes for the drug to reach its maximum accumulation in tumor, as determined by an independent method [41]. With Teraphthal introduced under these conditions, the therapeutic effectiveness of ultrasound action more than doubles, and the lifespan of mice increases by 30–40%; no toxic effects are detected. These conditions were subsequently selected for the combination therapy with ultrasound, Teraphthal, and cytostatics.

As analog of Teraphthal we tested octasodium salt of zinc octacarboxyphthalocyanine, which is not inferior in the sonosensitizing activity to Teraphthal while being less toxic. The dependence of the sonosensitizing activity on the drug dose over a wide range of nontoxic values is characterized by a curve with saturation. This allows obtaining more stable results [46].

Among Teraphthal derivatives we also tested sodium salt of monoconjugate of cobalt octa-4,5-carboxyphthalocyanine with folic acid, tetrasodium salt of tetraconjugate of cobalt octa-4,5-carboxyphthalocyanine with asparagine, Efiter[2], and cobalt phthalocyanine choline chloride. The choice of the Teraphthal conjugates was dictated by the fact that they can be accumulated in the tumor lesion, with specific receptors occurring on the tumor cell surface due to interaction [47–49].

[1] Ultrasonic alone of intramuscular transplanted tumors at elevated temperatures, compared to the temperature of the body, is often called ultrasound hyperthermia in literature. Given the specific features of ultrasound-induced heating of biological tissues and the multifactor nature of combined ultrasound therapy it would be more appropriate to refer to the temperature dependence of the effect examined, because ultrasonic absorption causes the temperature of the object to increase due to dissipation of acoustic energy by different routes (viscous, viscoelastic, shear, cavitation, radiation absorption, etc.). Different routes have different "destructive powers" and make different contributions to the overall increase in temperature. The sonosensitizer and cytostatic agents occurring in the system affect the energy dissipation routes, thereby making uncertain the "ultrasound hyperthermia term."

[2] An adduct of cobalt octacarboxyphthalocyanine acid tetra [methyl-penta-(hexa)-ethyleneglycol] ester with poly-cycloheptadextrin (RF patent 2172319, 2000).

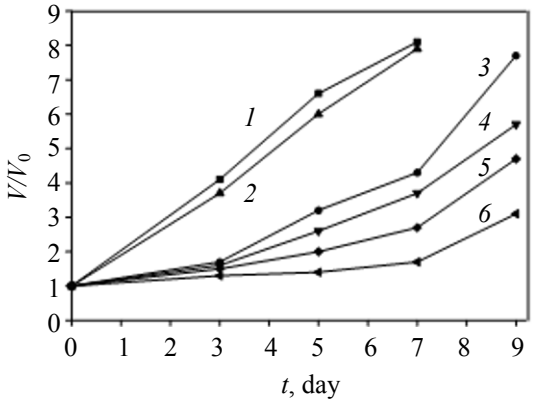

Fig. 8. Tumor growth dynamics under the combined action of ultrasound and hydroxyapatite nanoparticles with albumin sorbed (25 mg kg^{-1}) in relation to the time interval between the introduction of the nanoparticles and ultrasound exposure. Melanoma B16/F10 tumor. (*1*) Control, (*2*) nanoparticles, (*3*) ultrasound, and (*4, 5, 6*) nanoparticles + ultrasound after a (*4*) 15-min, (*5*) 1-h, and (*6*) 4-h interval.

Table 1 shows that the Teraphthal conjugates with asparagine and folic acid surpass in the sonodynamic activity the remainder of the sensitizers tested, from which they differ in the solid phase formation rate in acid and calcium media, as well as in the ability to form aggregates and in the adsorption capacity. Additional studies aimed to optimize the application conditions for these sonosensitizers and toxicity must show their competitiveness with respect to Teraphthal. As to Efiter and cobalt phthalocyanine choline chloride, they do not outperform Teraphthal in the sonosensitizing activity.

A drawback suffered by the method of synthesis of solid-phase inclusions (sonosensitizer) directly in the tumor consists in the fact that the solid phase mass depends on the physicochemical conditions in the tumor. For certain types of tumors it is at all impossible to synthesize the desired amount of the solid phase, in which situation the nanoparticles synthesized in vitro can be introduced in the bloodstream. The main problem with this method of introduction is the toxicity of nanoparticles.[3]

[3] Researchers tend, without any sound grounds, to optimistically report complete harmlessness and biocompatibility of drugs they propose, focusing on the obvious positive effect. At the same time, the toxicity of nanoparticles represents an acute problem which gradually becomes of prime importance in nanomedicine. Efforts and costs involved in addressing this problem even exceed those required for studying their functional (target) properties [50].

We suggested that hydroxyapatite, because of its biocompatibility, be used as solid-phase sonosensitizer. Also, there is evidence that hydroxyapatite naturally occurs in blood and is harmless when injected into the bloodstream by an invasive procedure [51, 52]. To reduce the risk of embolism, we developed a procedure for synthesis of highly dispersed hydroxyapatite in an ultrasonic field, followed by adsorption of albumin. Albumin is accumulated in solid tumors and can therefore be used both as a stabilizer of the solid phase and a means of its delivery to the tumor. The hydroxyapatite particles obtained under the above-descrybed conditions have an average size of 100–200 nm and are characterized by a fairly narrow size distribution.

It was found that the combined action of ultrasound and hydroxyapatite nanoparticles leads to pronounced superadditive inhibition of the tumor growth as dependent on the time interval between injection of the nanoparticles and ultrasound exposure. Our experiments reliably evidence doubling of effect from the combined action relative to the action of ultrasound alone (Fig. 8). Notably, the sensitizing activity of hydroxyapatite depends on the nature of its surface-bound compound. In the absence of albumin, hydroxyapatite exhibits negligible sonosensitizing activity. At the same time, it was shown that albumin-bound hydroxyapatite is able of sorbing a cytostatic agent (doxorubicin), in which case the sonosensitizer acts simultaneously as the drug container.

The experiments showed that, at pH 7 and 6, desorption of doxorubicin is negligible; it becomes noticeable at pH $\leq$ 5. Thus, using the approach proposed by us it is possible to obtain a hydroxyapatite complex with albumin and doxorubicin, stable at physiological pH values, which release the cytostatic agent under acidosis in tumor (increased acidity, pH = 5). It should be noted that the acidity of the tumor allows system regulation. This offers an additional opportunity for improving the efficacy of the combination therapy using ultrasound and sonosensitizers, with the latter acting simultaneously as a transport form for the cytostatic agent.

Combined Action of Ultrasound and Cytostatic Agents

In the preclinical study of the combined action of ultrasound and cytostatic agents we examined the influence exerted by ultrasound on the therapeutic efficacy of the following chemotherapeutic agents, both alone and combined: alkylating agents (sarco-

lysine, cyclophosphamide, ifosphamide, aranoza, platinum compounds); triazene drug (dacarbazine); mitotic inhibitors (taxoids, vincristine); antitumor antibiotics (doxorubicin); and antimetabolites (5-fluorouracil, gemcitabin, methotrexate).

It was found that, under close to optimal ultrasonication conditions, the enhancement ratios for ultrasound action combined with the therapeutic effect of cytostatic agents are identical, 3–5, on the average. This is due, presumably, to the changes in the effective diffusion coefficient of the cytostatic agents under ultrasound exposure, caused by the change in the physicochemical characteristics of the tumor tissue, which leads to increased bioavailability of the drugs. In each specific case the efficacy changes differently, depending on the structure of the cytostatic agent molecule, determining the rate of its delivery to the target under conditions of an altered tumor structure.

We examined the therapeutic effect of ultrasound in combination with the basic chemotherapy schemes applied in clinical practice: doxorubicin + cisplatin, Aranoza + cisplatin, adriablastin + carboplatin, and doxorubicin + dacarbazine. Ultrasound exposure caused a 3–5-fold increase in the efficacy of these treatment schemes, which is nearly identical to that achieved with individual cytostatics.

Two courses of therapy provide significantly better results than one course. For example, in the case of a combination of intravenously injected adriablastin (4 mg/kg) with intraperitoneally injected carboplatin (30 mg/kg), the tumor volume doubling time in one course of sonodynamic therapy was 8 days, and in two courses with a 2-day interval between the courses, 14 days. Against this backdrop, the data from one and two courses of monotherapy with ultrasound differ only slightly, being indicative of a superadditive therapeutic effect and calling for detailed examination of this phenomenon [53–55].

Of special note is the capacity of ultrasound to overcome the induced drug resistance, the main factor decreasing the chemotherapy efficiency. As already demonstrated for a cell culture (see above), multidrug resistance can be overcome by ultrasound. Similar results were provided by the in vivo experiments, involving dual-frequency ultrasound exposure. For example, intraperitoneally at the 300 mg/kg dose had virtually no effect on the growth of melanoma B16/F10 with doxorubicin resistance (B16/DC), thereby confirming the resistance of the tumor to this drug.

Table 1. Sonosensitizing action of Teraphthal and its derivatives

Sonosensitizer	Enhancement ratio for the antitumor effect relative to the control
Teraphthal	4.5–5
Octasodium salt of zinc octacarboxyphthalocyanine	5–5.5
Efiter	4–4.5
Phthalocyanine choline cobalt chloride	3.5–4
Conjugate of cobalt octacarboxyphthalocyanine with asparagine	9–10
Conjugate of cobalt octacarboxyphthalocyanine with folic acid	8
Ultrasound	1.5–2

When applied alone, ultrasound had negligible effect on the tumor growth as well. At the same time, experiments with a dacarbazine + ultrasound combination provided a reliable evidence that the inhibitory effect produced on the tumor growth was enhanced by a factor of 2.6 compared to the control [45].

Combined Action of Sonodynamic Therapy and Cytostatic Agents

The ultrasound + sonosensitizers and ultrasound + cytostatic agents combinations produce a different therapeutic effects. In this connection, a question arises, to what extent it is advisable to use a complex combination ultrasound + sonosensitizer + cytostatic. It should be borne in mind that increases in ultrasound intensity, temperature in the tumor, and doses of sonosensitizers and cytostatic agents cause enhanced inhibition of the tumor growth. At first glance, there is no need in a complex combination. However, increased ultrasound intensity causes irreversible changes in healthy tissue, and increased temperature and doses, enhanced toxic action and long-term adverse effects.

The choice of therapeutic scheme is dictated by the specific pathology. The selection criterion is the condition under which stabilization of the tumor growth, or remission, are achieved with minimum adverse effects. In some cases, an ultrasound + cytostatic agent combination is sufficient. However, there are cases where inclusion in the therapeutic scheme of a nontoxic sonosensitizer significantly improves the

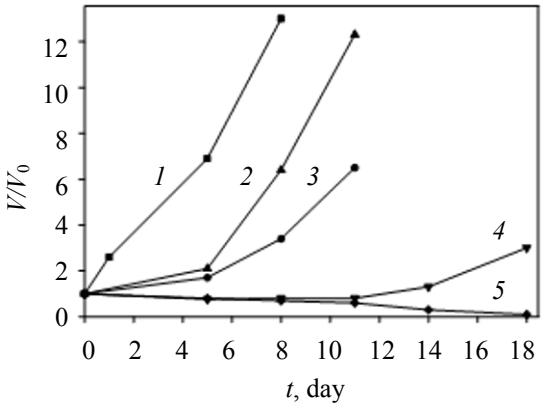

Fig. 9. The Ca755 growth dynamics under the combined action of ultrasound, Taxotere, and Teraphthal: (*1*) control, (*2*) ultrasound, (*3*) Taxotere (20 mg/kg), (*4*) Taxotere + ultrasound, and (*5*) Taxotere + Teraphthal (20 mg/kg) + ultrasound.

result. An example can be found in the ultrasound + cytostatic Taxotere + Teraphthal treatment schedule.

Figure 9 shows that, when applied as monotherapy at the average therapeutic dose of 20 mg/kg, Taxotere is highly efficacious toward treating established Ca775 tumor, as evidenced by enhancement ratio K = 11, complete remission (CR) rate 25%, and satisfactory tolerability of treatment; no side effects or death from toxicity were recorded. When ultrasound was applied 3 h after injection of Taxotere, the treatment efficacy was significantly improved in both parameters: K = 31, CR = 50%. The complete sonodynamic therapy schedule (Taxotere + Teraphthal + ultrasound) was the most efficacious and led to CR = 100% in all the animals used in our experiments. The treatment tolerability proved to be satisfactory; no side effects or death from toxicity were recorded.

Thus, a treatment session with Taxotere at the dose of 20 mg/kg, Teraphthal at the dose of 20 mg/kg, and ultrasound exposure in the standard regime (2- and 1-h intervals, respectively) leads to complete regression of Taxotere-sensitive established mammary adenocarcinoma Ca755 with satisfactory tolerability.

The experiments on the mice with intramuscularly LLC and on the rats with polymorphic cell sarcoma (clear cell sarcoma) also showed that tumor regression until full recovery can be achieved only via application of ultrasound in combination with chemotherapy and Teraphthal. The latter increases the efficacy of ultrasound and chemotherapy without affecting the general and local tolerability.

These results can be regarded as adequately substantiating the inclusion of sonosensitizers (compounds that do not exert a cytotoxic effect) into in the therapeutic schedule for continuation of experiments in vivo.

Toxic Effects from the Combined Action of Sonodynamic Therapy and Cytostatic Agents

For each specific schedule of combination therapy with the use of ultrasound it is necessary to estimate experimentally the upper temperature limit as determined by the ultrasound intensity and exposure time, beyond which the toxicity becomes unacceptable.

As demonstrated by our in vivo experiments, the use of ultrasound at 44–45°C in combination with cisplatin causes strong general toxic and local effects. At 42°C the specific toxicity was significantly less pronounced, and at 41°C, negligible. Multiple sessions at 41°C also do not cause changes in the physiological condition and behavior of the animals and in all clinical parameters (blood and urine values, electrocardiogram, weight coefficients of internal organs, etc.). Therefore, when ultrasound is applied in combination with cytostatic agents it is necessary either to decrease the cytostatic agent concentration or to lower the temperature to 41°C. Specifically at 41°C we performed toxicological examinations of the therapeutic schedules, promising for clinical studies, on healthy rats and dogs.

Earlier [55], we revealed satisfactory tolerability of locally applied ultrasound combined with Teraphthal in chemotherapy with the use of anticancer cytostatic agents acting by different mechanisms. No pronounced side effects were detected. For the regimes used in our experiments (41°C, standard doses of cytostatic agents and Teraphthal) no changes were revealed in behavioral responses and body weight and temperature of the animals, as well as in the morphofunctional parameters of their cardiovascular system, liver, kidneys, and other organs. Thus, the conditions we selected for the combination treatment using chemotherapy, ultrasound, and sonosensitizer Teraphthal can be recommended for clinical studies.

Effect on Metastasis Formation

High lung-metastatic melanoma B16/F10 was trans-planted to the muscles of the leg of the animals. One group of animals served as control, and other groups received single treatment of combined chemotherapy with adriablastin + carboplatin, Teraphthal + ultrasound, or ultrasound alone. The main

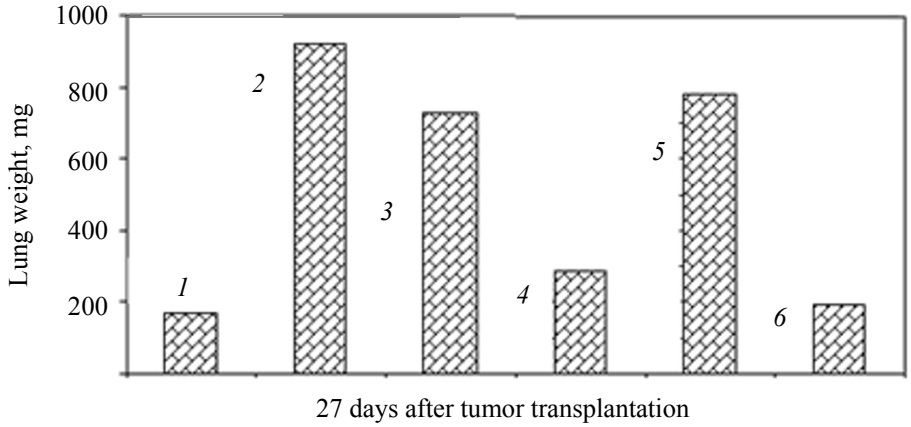

Fig. 10. Weight of lung metastases of melanoma B16 after combined therapy with ultrasound therapy with adriablastin, carboplatin, and Teraphthal (TPh). (*1*) Healthy tumor-non-bearing mice, (*2*) melanoma B16, primary tumor removed, (*3*) ultrasound, (*4*) Adriablastin + Carboplatin, (*5*) TPh + US, and (*6*) Adriablastin + Carboplatin + TPh + US.

group received combined therapy with adriablastin + carboplatin + Teraphthal + ultrasound. For conducting univariate analysis, the primary tumor was removed 24 h after the exposure. To determine the degree of lung metastasis, the lung mass from the treated mice was compared with that from the control group (untreated) and from the group of healthy mice (Fig. 10).

The comparative studies showed that, in treatment of the mice with melanoma B16, the adriablastin + carboplatin + Teraphthal + ultrasound combination does not stimulate lung metastasis. Exactly the opposite, this therapy causes the weight of the lungs with metastases to decrease. No promotion of metastases was also observed in the case of individual chemotherapy or for Teraphthal + ultrasound combination. In the groups of the mice that received treatment by these schemes the weight of the lungs with metastases is smaller than that in the untreated mice.

Mechanism of Therapeutic Effect

To elucidate the action mechanism for combined ultrasound therapy, the pathomorphosis in melanoma after combined therapy with ultrasound, Teraphthal, and carboplatin was examined histo-logically. The examination was performed at the Department of Pathological Anatomy [proff. N.T. Raikhlin].

Table 2 lists the results from the combined use of the therapy components.

The histological and electron-microscopic examinations showed that ultrasound causes extensive injuries in the blood vessels and massive hemorrhages of the tumor, reduces the mitotic activity of the tumor cells, slightly enhances apoptosis, and decreases the invasive capacity (Fig. 11).

In the tumor cells and in the vascular endothelium, mitochondria are injured. Specifically, broken cristae (membrane structures) are observed, and mitochondria are subjected to lysis and disintegration, which ultimately promote cell death as well. Ultrasound causes replacement of active euchromatin by inactive condensed heterochromatin in the nuclei of the tumor cells. These changes are observed already 3 h after exposure to ultrasound and are enhanced in the subsequent 48 h.

With ultrasound, Teraphthal, and carboplatin used simultaneously, the changes detected in the tumor are enhanced relative to ultrasound applied alone or in combination with each of these drugs separately.

Thus, ultrasound exposure causes injuries to a part of the existing circulatory system in tumor and also may prevent neoangiogenesis, which plays a leading role in the progression of neoplasms (invasion, recurrence, metastasis, growth rate, etc.).

An immunohistochemical examination showed that ultrasound exposure under the experimental conditions not only causes injuries to the blood vessels, plasma membranes, mitochondria, and other organelles of the tumor cells but also inhibits neoangiogenesis in the tumor areas that remained intact, thereby preventing the continued growth of the neoplasm. When ultrasound is combined with Teraphthal, their individual effects are summarized and even slightly enhanced. There is a reason to believe that combinations of ultrasound with other cytostatic agents and sonosensitizers act by a similar mechanism.

Table 2. Histological changes in the tumor (melanoma B16) under the action of ultrasound and ultrasound + Teraphthal + carboplatin combination

Changes	Control (series I)	Ultrasound (series II)	Ultrasound + Teraphthal (series III)	Ultrasound + carboplatin (series IV)	Ultrasound + Teraphthal + carboplatin (series V)
Area of necroses, %	5–8	30–40	40–50	40–50	50–60
Mitoses, %	2.0–3.0	1.5–2.0	1.0–1.5	1.0–1.5	0.5–1.0
Apoptosis, %	0.2–0.3	0.2–0.5	0.3–0.5	0.2–0.5	0.3–0.5
Development of vascular injuries in tumor	Lacking	Partial (up to 1/3)	Partial (up to 2/3)	Partial (up to 2/3)	Subtotal
Hyperemia in tumor	Moderate	Strong	Very strong	Very strong	Very strong
Hemorrhages in tumor	Few	Many	Abundant	Abundant	Abundant
Invasion	Pronounced	Moderate	Reduced	Reduced	Reduced
Swelling of adjacent tissue	Weak	Moderate	Moderate	Moderate	Moderate
Hemorrhages in adjacent tissue	Few	Many	Many	Many	Many
Muscle tissue dystrophy and lysis	Weak	Strong	Strong	Strong	Strong

In all the experiments on combined use of Teraphthal, cytostatic agents, and ultrasound the enhanced therapeutic efficacy was achieved without enhancement of the toxic action. This finding gives certain advantages to this combination over ultrasound combined with cytostatics as sole agents. In some cases, the overall efficacy of the Teraphthal + cytostatic agent + ultrasound combination is simply the sum of the individual effects from the components, and in other cases, a superadditive effect is achieved. We already showed (see above) that the use of ultrasound in combination with Teraphthal leads to destruction of the tumor cell membrane structures and organelles. This destruction both causes the death of the cell and leads to a situation in which the cell can restore its integrity, but the permeability of the membranes is significantly increased after ultrasound exposure. Thereby, additional possibilities arise for transporting cytostatic agents to the target, and this is specifically responsible for therapeutic superadditivity.

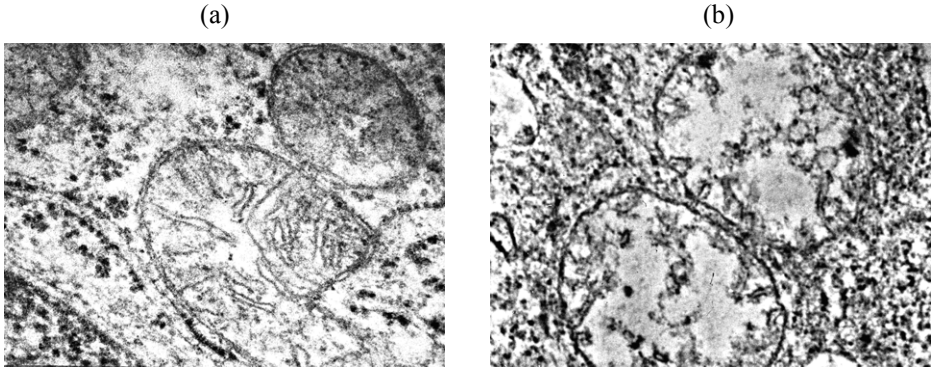

Fig. 11. Electron micrographs of the mitochondria of melanoma B16 cells: (a) control and (b) tumor after ultrasound exposure in the presence of Teraphthal.

Additional Options for Improving the Ultrasound Therapy Efficacy

The efficacy of combined ultrasound therapy can be increased by locally decreasing the cavitation resistance of the medium in the tumor, thereby intensifying the cavitation processes responsible for destruction of the tumor cells. The cavitation resistance of the medium depends substantially on the dissolved gas concentration. The carbon dioxide level in blood and tissues can be adjusted within certain limits by introducing a sodium bicarbonate solution (used in medical practice as a remedy for acidosis). Sodium bicarbonate injecttions to animals should cause a temporary increase in the carbon dioxide concentration in the tumor and, thereby, a decrease in its cavitation resistance. In model experiments, mixing of weak organic acids with sodium bicarbonate in an acoustic field led to a burst of cavitation, as detected from an increase in the subharmonic intensity (f = 0.44 MHz).

In a real living system, an excess of sodium bicarbonate injected triggers additional compensatory mechanisms (hyperventilation, etc.). However, nonsteady-state conditions can exist for a certain period during which, upon application of an ultrasonic field, the local intensity of the cavitation processes in the tumor should increase, thereby enhancing the therapeutic effect. The suggestions made were confirmed in experiments on animals in which ultrasound was applied both as monotherapy [56, 57] and in combinations with cytostatic agents and sonosensitizers.

The efficacy of combined ultrasound therapy of tumors can be further enhanced by decreasing the strength and increasing the permeability of the membrane structures and the vascular system of tumor, e.g., by significantly increasing the amount of polyunsaturated fatty acids (like omega-3) in the diet. Polyunsaturated acids are rapidly incorporated into the tumor cell membranes and cause a decrease in the content of saturated fatty acids, providing the cell membrane carcass rigidity [58].

Also suitable for this purpose are cholesterol synthesis inhibitors, since cholesterol is a component essential for strengthening the membrane structures.

Clinical Studies

For clinical examination of the efficiency of combined ultrasound therapy, a setup for sonodynamic therapy was designed, manufactured, and registered by the Federal Service for Supervision in the Field of Health Care and Social Development (Fig. 12).

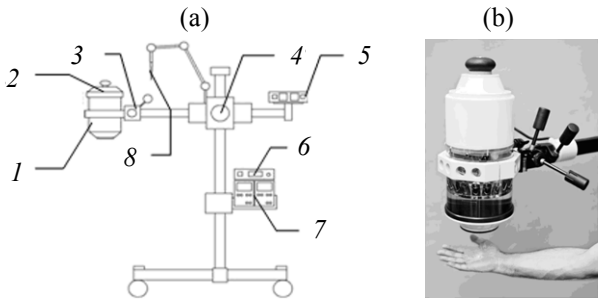

Fig. 12. Setup for clinical trials of ultrasound-based combination therapy. (a) Setup schematic: (*1*) immersion tank, (*2*) acoustic system, (*3*) device for mounting the acoustic system, (*4*) positioner tripod, (*5*) thermal unit, (*6*) control unit (*7*) power supply unit, and (*8*) temperature sensor and (b) acoustic system.

The acoustic system of the setup consists of 16 emitters providing emission at two frequencies simultaneously, 0.88 and 2.64 MHz, and a laser system for "targeting" the irradiation unit at the selected sector of the tumor site. The maximum effective penetration depth is 8 cm. The setup is equipped with a complex unit for controlling the operating parameters: the radiation intensity and frequency, the temperature in the exposed area, the duty factor, the time of exposure, and the vertical scan speed and amplitude.

In its operating mode the setup provides for tumor surface scanning along the sectors selected under conditions of continuous-wave ultrasound exposure in a way such that exceeding of the temperature set-point in the tumor prevented. The technique applied combines the vertical scan of the tumor and two-point temperature control with the feedback and thereby allows optimizing the distribution of acoustic energy in the tumor volume and minimizing the adverse effects associated with tumor inhomogeneity.

The Department of General Oncology, Research Institute of Clinical Oncology, RONTs, initiated studies on combined ultrasound therapy within the framework of the clinical trial protocol developed. Treatment of patients with soft-tissue sarcomas localized in the zone accessible to ultrasound involved ultrasound exposure combined with chemotherapy.

The observation conducted in accordance with the protocol revealed no early side effects. Ultrasonic treatment in the regimes employed is satisfactorily tolerated and causes no local or systemic complica-

tions in patients, which could limit its use. Combined with ultrasound, chemotherapy was tolerated satisfactorily; no increase in chemotherapy toxicity was detected. Treatment sessions combining ultra-sound exposure with chemotherapy do not create the need for adjustment of the dose of cytotoxic drugs, extension of the rehabilitation term, or cessation of the therapy.

The result of the therapy by the method proposed was that two patients had more than 30% of shrinkage of their tumor, and one patient had an effect that was qualified as progression. In all other cases, stabilization was observed. Operative therapy was performed on 8 of 9 patients.

Clinical trials were initiated for the combined method including chemotherapy and ultrasound with sonosensitizer Teraphthal. Treatment of patients with different localizations of disseminated skin melanoma was carried out in accordance with the protocol.

Studies of the treatment schemes showed that the method of sonodynamic therapy with Teraphthal and scheduled chemotherapy (cisplatin, doxorubicin, dacarbazine) cause no early and late side effects. The combination of ultrasound, Teraphthal (3 mg m^{-2}), and scheduled chemotherapy provides long-term stabilization of tumor in 80% of cases against background of the overall progression of the process. It should be noted that the clinical trials are in early stage now and that low Teraphthal doses were used in the treatment schemes employed. Further clinical trials will employ higher Teraphthal doses (no lower than 10 mg m^{-2}) as preceded by dose escalation.

Thus, positive results from application of the combined method of ultrasound therapy with cytostatic agents in treatment of soft-tissue sarcoma and that with cytostatic agents and Teraphthal in treatment of disseminated melanoma justify further extended clinical studies of the method as applied to other types of tumors. Issues to be addressed include optimization of the doses of the components and intervals between their injections, as well as of the ultrasound exposure regimes, properties of sonosensitizers, etc. While representing just one of the possible choices, combined method of ultrasound therapy holds promise for greatly improving the effectiveness of existing treatment methods.

CONCLUSIONS

Laboratory studies and preclinical trials showed that the combined method of ultrasound therapy of malignant tumors with sonosensitizers and cytostatic agents allows enhancing the injury to the tumor, caused by ultrasound, while no metastasis-promoting and toxic effects are exerted under the conditions chosen. The bioavailability of therapeutic agents to the tumor, in particular to cells exhibiting multidrug resistance, increases.

Of particular interest are complex pulse ultrasound regimes, whose application allows, first, reducing the total radiation dose and decreasing the pain, and, second, achieving the so-called surface resonance. The latter effect can serve as an additional factor affecting the tumor cells.

Correct choice of noncytostatic supporting therapy scheme is of great importance. In the case of intravenously injected solutions of solid-phase sonosensitizers producing a solid phase of acid forms directly in the tumor, a favorable factor consists in systematic stimulation of acidification of the tumor.

Enhanced therapeutic efficacy was achieved in experiments on the mice with intramuscularly transplanted melanoma B16, subjected to ultrasound exposure with introduction of Teraphthal and glucose. We attributed this observation to an increase in the effective dose of the solid phase of the acid form of Teraphthal in the tumor due to acidification of the latter, caused by introduction of glucose.

Thus, the combined method of ultrasound therapy of malignant tumors was experimentally justified and instrumentally implemented at the preclinical and clinical levels. In our opinion, the suitability for addressing the above-listed problems will allow this method to become equal in clinical practice to photodynamic therapy and radiotherapy of cancer individually and, possibly, in combination with these methods.

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